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Date: Dec. 17, 2001 By: Lois E. Miller  
Lois E. Miller

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

J. Fernando BAZAN, et al.

Serial No.: 09/963,347

Filed: September 25, 2001

For: MAMMALIAN CYTOKINES;  
RELATED REAGENTS AND  
METHODS

Examiner: not yet assigned

Art Unit: not yet assigned

PRELIMINARY AMENDMENT

Palo Alto, California 94304

Dec. 17, 2001

Box Missing Parts  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Honorable Sir:

Please amend the Specification of the above-identified case to correctly refer to

the amended formal Figures and correct errors in the specification.

AMENDMENT

IN THE SPECIFICATION:

Please replace the paragraph beginning at page 4, line 8 with the following rewritten paragraph:

5       -- Figures 2A-2E show expression levels of hIL-7R $\alpha$  (Figures 2A-2B), R $\delta$ 2 (hTSLPR, Figures 2C-2D), and IL-B50 in various tissues and cell types. Expression levels were normalized and expressed as femtograms mRNA per 50 ng total cDNA. --

10       Please replace the paragraph beginning at page 4, line 26 with the following rewritten paragraph:

      -- Figures 6A-6C show the surface phenotype of DC after treatment with medium alone, IL-B50, CD40-ligand (CD40L), IL-7 and LPS. IL-B50 is more potent than CD40-ligand and IL-7 in upregulating costimulatory molecules CD40 and CD80. --

15       Please replace the paragraph beginning at page 64, line 18 with the following rewritten paragraph:

20       -- In order to identify target cells capable of responding to IL-B50888888, a large panel of cDNA libraries was analyzed for the simultaneous expression of both hIL-7R $\alpha$  and hR $\delta$ 2, using quatitative PCR. Results of the expression analysis, conducted as described in materials and methods, are presented in Figures 2A-2E. In particular, expression analysis of the two receptor subunits indicated that they were co-expressed primarily in activated dendritic cells, monocytes, and T cells (see, Figures 2A-2D) indicating that these cell types respond to human IL-B50. As shown in Figure 2E, IL-B50 was expressed in various tissue types, with high expression in the human lung. --

Please replace the paragraph beginning at page 65, line 15 with the following rewritten paragraph:

- - Additionally, the ability of IL-B50 to stimulate DCs to produce mRNAs for various cytokines and chemokines was compared with that of GM-CSF, IL-7, CD40-ligand (CD40L) and medium alone as a control, as follows. Purified CD11c+ DCs were cultured for 15-17 hours with IL-B50 (15 ng/ml), GM-CSF (100 ng/ml), IL-7 (50 ng/ml), CD40-ligand transfected L-cells (1 L-cell/4 DC) or medium alone. Total RNA was extracted and studied using real time quantitative PCR as described above. As shown in Figures 12A and 12C, IL-B50 did not stimulate human DCs to produce mRNA for IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-12p40, TNF- $\alpha$ , MCP-1, MCP-4, Rantes and MIG, but did stimulate human DCs to produce mRNA for the chemokines TARC, MDC and MIP3- $\beta$  (Figure 12B). - -

Please replace the paragraph beginning at page 67, line 9 with the following rewritten paragraph:

- - Freshly purified immature CD11c+ blood DC are known to spontaneously mature in culture. As shown in Figure 4A, loose and irregular clumps in the DC culture were evident after 24 hrs in medium alone. In the presence of IL-B50, this maturation process was dramatically enhanced. DC in culture formed tight and round clumps with fine dendrites visible at the periphery of each clump (Figure 4B). The IL-B50-induced maturation was confirmed by analyzing the surface phenotype of DC using flow cytometry. Whereas IL-B50 slightly upregulated the expression of HLA-DR and CD86, it strongly induced the costimulatory molecules CD40 and CD80 (Figure 5). This

maturation process was accompanied by an increased viability of the DC. Additionally, IL-B50 was more potent than CD40-ligand (CD40L) and IL-7 in upregulating CD40 and CD80 (Figures 6A-6C). A titration of IL-B50 using log dilutions of the cytokine showed that both the effect on survival and the induction of costimulatory molecules on DC was maximal at 15 ng/ml and above, and still significant at concentrations as low as 15 pg/ml. - -

#### REMARKS

Applicants respectfully request these amendments be entered to properly refer to Figures 2A-2E, and Figures 6A-6C, rather than the original reference to Figures 2A-2C and a single Figure 6. A typo is corrected on page 67, at line 14, to correctly reference Figure 4B, and not 6B. Applicants also seek to correct the failure of Greek characters to print on page 65, beginning at line 22.

Applicants believe that no new matter is added by way of this amendment.

Attached hereto is a marked up version of the changes made to the specification by the current amendment. The attached page is captioned, "Version with markings to show changes made."

#### Summary

Applicants' amendments are necessary due to formalization of Figures 2A-2E, and Figures 6A-6C, and to correct the reference to Figure 4B. Greek characters that failed to print on page 65, beginning at line 22, are corrected.

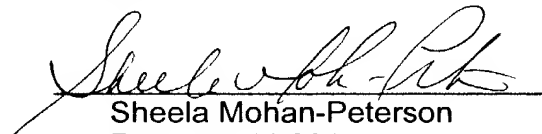
CONCLUSION

If there exist any issues with the current amendment, Applicants respectfully request that an interview be granted with the undersigned attorney to discuss any remaining issues of problems with the foregoing amendment. The Examiner is invited to telephone the undersigned at (650) 496-1244 to arrange for a mutually convenient time and form for the interview.

Applicants believe that no fees are required; however, if any fees are required by the present Response, the Commissioner is authorized to charge any fees or credit any overpayment to DNAX Research Institute Deposit Account No. 04-1239.

Respectfully submitted,

December 17, 2001

  
Sheela Mohan-Peterson  
Reg. No. 41,201

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901 California Avenue  
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**VERSION WITH MARKINGS TO SHOW CHANGES****In the Specification:**

5

Paragraph beginning at line 8 of page 4 has been amended as follows:

10 Figures 2A-[2C]2E show expression levels of hIL-7R $\alpha$  (Figures 2A-2B), R $\delta$ 2 (hTSLPR, Figures 2[B]C-2D), and IL-B50 in various tissues and cell types. Expression levels were normalized and expressed as femtograms mRNA per 50 ng total cDNA.

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Paragraph beginning at line 26 of page 4 has been amended as follows:

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Paragraph beginning at line 15 of page 65 has been amended as follows:

Additionally, the ability of IL-B50 to stimulate DCs to produce mRNAs for various cytokines and chemokines was compared with that of GM-CSF, IL-7, CD40-ligand (CD40L) and medium alone as a control, as follows. Purified CD11c+ DCs were cultured for 15-17 hours with IL-B50 (15 ng/ml), GM-CSF (100 ng/ml), IL-7 (50 ng/ml), CD40-ligand transfected L-cells (1 L-cell/4 DC) or medium alone. Total RNA was extracted and studied using real time quantitative PCR as described above. As shown in Figures 12A and 12C, IL-B50 did not stimulate human DCs to produce mRNA for [IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-12p40, TNF- $\alpha$ , MCP-1, MCP-4, Rantes and MIG, but did stimulate human DCs to produce mRNA for the chemokines TARC, MDC and [MIP3- $\alpha$ ] MIP3- $\beta$  (Figure 12B).

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Freshly purified immature CD11c+ blood DC are known to spontaneously mature in culture. As shown in Figure 4A, loose and irregular clumps in the DC culture were evident after 24 hrs in medium alone. In the presence of IL-B50, this maturation process was dramatically enhanced. DC in culture formed tight and round clumps with fine dendrites visible at the periphery of each clump (Figure [6]4B). The IL-B50-induced maturation was confirmed by analyzing the surface phenotype of DC using flow cytometry. Whereas IL-B50 slightly upregulated the expression of HLA-DR and CD86, it strongly induced the costimulatory molecules CD40 and CD80 (Figure 5). This maturation process was accompanied by an increased viability of the DC. Additionally, IL-B50 was more potent than CD40-ligand (CD40L) and IL-7 in upregulating CD40 and CD80 (Figure[s] 6A-6C). A titration of IL-B50 using log dilutions of the cytokine showed that both the effect on survival and the induction of costimulatory molecules on DC was maximal at 15 ng/ml and above, and still significant at concentrations as low as 15 pg/ml.